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QUANTITATIVE EVALUATION OF DIFFERENT DOSAGE FORMS OF AMPICILLIN

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ABSTRACT

The bioavailability of five different commercial brands of ampicillin was examined. The absorption of each dosage form was compared in a crossover study of twelve healthy volunteers (6 males and 6 females). Urinary excretion rates were also employed to evaluate the absorption process. Statistical analysis of the results was carried out to evaluate the significance of differences between dosage forms and subjects. The statistical analysis indicated no significant differences between different tested brands of ampicillin, while the differences between subjects were significant. Comparison between the two different genders indicated no significant differences between the male and female subjects.

INTRODUCTION

It is well known that all commercially available products do not demonstrate equivalent bioavailability. Therefore, the evaluation of the bioavailability of various solid dosage forms is necessary. The

assessment of bioavailability of various solid dosage forms is especially valuable in countries where the pharmaceutical industry is less established and in countries which only have generic products.

Ampicillin is a very potent antibiotic and is used widely in serious infections with minimal toxicity and side effects. It has also been shown that ampicillin dosage forms exhibit differences in bioavailability (1,2). These differences, along with other conflicting reports (3,4,5), concerning the bioavailability of ampicillin from dosage forms made by different manufacturers, are the main reasons for selection of this drug in the present study.

After administration of ampicillin, more than 90% of the absorbed drug is excreted in the urine (6). Therefore, the total urinary recovery of the drug is an indication of the extent of ampicillin absorption. Bioavailability studies of ampicillin can be based on urine data (6-14) as well as the blood data (15,16). As the urinary excretion method is easier for the subjects, the comparative bioavailability of five brands of ampicillin capsules and tablets were assessed in a crossover study using twelve subjects.

MATERIALS and METHODS

Analytical grade copper sulfate, citric acid, disodium hydrogen phosphate, and trichloroacetic acid were used. The following different brands (A,B,C,D,E) of ampicillin capsules and tablets were selected:

Brand A, locally made using pure trihydrate ampicillin (98.2% on bases of Ampicillin Trihydrate) was used as the standard; brands C, D,

E and B (the only tablet form used) were manufactured by different local pharmaceutical companies.[1]

Tweleve healthy normal adult volunteers composed of; six males and six females were chosen. Their age ranged from 23-33 years (average 29.2 years) and weights between 52-65 kg (averages 60.6 kg). All subjects were examined by a hospital physician prior to the study. Also, each subject had no concurrent drug treatment for several days before and during the study.

Following an overnight fast, a single 500 mg capsule (or tablet) or two 250 mg capsules were administered with 250 ml of water. Following administration, no food or liquid were permitted for four hours except for 200 ml of water every hour. Urine samples were collected quantitatively at 1, 2, 3, 4, 6, 8 and 12 hours after drug administration. control samples were collected for 1 hour prior to drug administration.

The urine samples were kept at 4°C until analyzed the next day.

Ampicillin concentrations in collected urine samples were measured according to the method proposed by Angelucci and Baldieri (17). The method was reproducible and sensitive for the urine samples. A five point calibration curve was used throughout the analysis. Urine samples were diluted where needed.

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1 B: tablets made by Pars Daro Pharmaceutical Co.
C: capsules made by Daro-Pakhsh Pharmaceutical Co.
D: capsules made by Cowsar Pharmaceutical Co.
E: capsules made by Towlid-Daro Pharmaceutical Co.

RESULTS

Due to first order elimination kinetics, there is a relationship between blood level of ampicillin and its rate of urinary excretion (18). Therefore, urine data can be used to evaluate some of the pharmacokinetic parameters of ampicillin.

Cumulative Amount of Drug Excreted

To insure complete renal excretion of ampicillin, urine samples were collected for 12 hours after drug administration. Because of rapid absorption and elimination of ampicillin in normal healthy adult volunteer, the urine samples collected over this period is sufficient (15).

Cumulative urinary excretion data for drugs which are eliminated in the unchanged form through the kidneys is a valuable parameter describing their bioavailability (19). To ensure complete clearance of the drug the urine was collected for 12 hours. Since the concentration of ampicillin in the urine sample collected at 12 hours showed negligible value, therefore the cumulative amount excreted after 8 hours would be a proper indication of the extent of ampicillin absorption. The cumulative amount of ampicillin excreted over a period of 8 hours after administration of various brands are shown in Fig. 1. Pharmacokinetic parameters for brands and for subjects are shown in tables 1 and 2 respectively. Table 1 indicates that the average amount of ampicillin excreted 8 hours after administration of all different dosage forms is 176.5 mg (range from 153.3 to 189.6).

In Table 2 the average amount of drug excreted over the same period of time after administration to twelve subjects can be

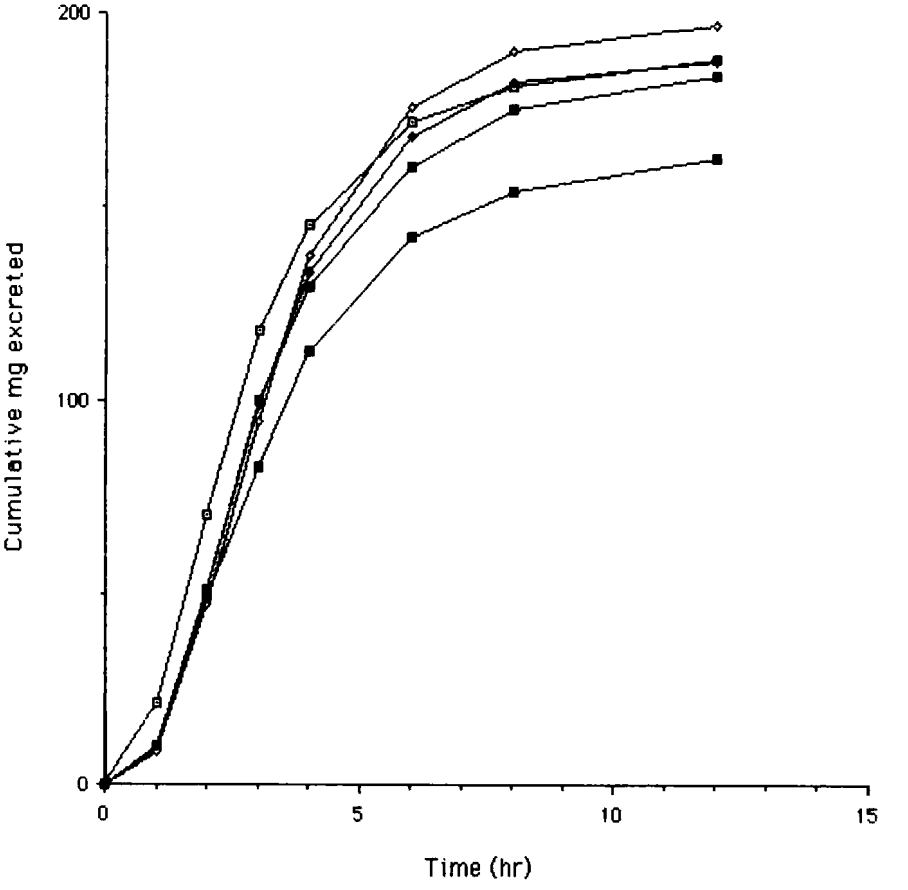

FIGURE 1

Average cumulative ampicillin excreted into urine following oral administration of the following ampicillin dosage forms to twelve subjects: ⊡, brand A; ◆, brand B; ■, brand C; ◇, brand D; ■, brand E.

observed. The average amount excreted is 176.5 mg (range from 144.6 to 220.5 mg). Analysis of variance (10) of these data indicates that there are no significant statistical differences (p=0.05) between the different brands and different subjects.

TABLE 1

Average Pharmacokinetic Parameters Per Brand Following Oral Administration of 500 mg of Ampicillin in Different Dosage Forms (4 Capsules and One Tablet) to Twelve Subjects (6 Males, 6 Females).

Brand (Capsules)	Cumulative (mg) Excreted after 8 hr	Urinary Peak height (mg/hr)	Time of Peaking (hr)	Ke[a] (hr^{-1})	t1/2[b] (hr)
A[c]	181.8 (14.9)[d]	62.0 (6.7)	2.00 (0.15)	0.524 (0.023)	1.32
B[e]	181.8 (16.7)	56.3 (4.8)	2.20 (0.16)	0.492 (0.028)	1.41
C	175.9 (17.3)	53.7 (6.1)	2.10 (0.13)	0.492 (0.028)	1.41
D	189.6 (12.2)	51.0 (4.4)	2.30 (0.22)	0.450 (0.022)	1.54
E	153.3 (9.6)	48.0 (3.0)	2.20 (0.17)	0.468 (0.022)	1.48
Mean	176.5 (6.2)	54.2 (2.4)	2.16 (0.05)	0.485 (0.012)	1.43
Statistical Analysis	N.S.[f]	N.S.	S.[g]	N.S.	N.S.

a elimination rate constant
b harmonic mean of half-life
c used as standard
d numbers in parenthesis represent the standard error of the mean
e the only tablet dosage form
f not significant (p=0.05)
g significant (p=0.05)

TABLE 2

Average Pharmacokinetic Parameters Per Subjects Following Oral Administration of 500 mg of Ampicillin in Different Dosage Forms (4 Capsules and One Tablet) to Twelve Subjects (6 Males, 6 Females).

Subject	Cumulative (mg) Excreted after 8 hr	Urinary Peak height (mg/hr)	Time of Peaking (hr)	Ke[a] (hr^{-1})	t1/2[b] (hr)
ME	151.1 (15.9)[c]	48.7 (10.5)	1.94 (0.22)	0.411 (0.023)	1.69
JA	149.7 (10.3)	58.6 (7.5)	1.96 (0.22)	0.546 (0.036)	1.27
DA	180.4 (17.7)	62.2 (6.1)	2.42 (0.31)	0.532 (0.035)	1.30
AM	205.3 (21.1)	51.7 (6.4)	1.86 (0.29)	0.368 (0.030)	1.88
GO	195.1 (22.4)	61.9 12.9)	1.98 (0.18)	0.468 (0.034)	1.48
HO	220.5 (38.3)	63.4 (11.9)	2.56 (0.42)	0.502 (0.050)	1.38
NK+[d]	167.1 (21.3)	49.4 (6.2)	1.48 (0.49)	0.475 (0.022)	1.46
MO+	171.4 (23.8)	48.8 (3.5)	1.90 (0.17)	0.536 (0.030)	1.29
SH+	207.2 (14.9)	64.7 (8.3)	2.32 (0.21)	0.527 (0.015)	1.31
SA+	172.4 (24.9)	46.6 (4.7)	2.60 (0.24)	0.549 (0.093)	1.26
HE+	144.6 (16.5)	42.2 (1.8)	2.22 (0.20)	0.439 (0.032)	1.58
KO+	153.0 (7.3)	52.4 (10.0)	2.44 (0.17)	0.486 (0.033)	1.43
Mean	176.5 (7.3)	54.2 (2.4)	2.14 (0.10)	0.486 (0.016)	1.43
Statistical Analysis	N.S.[e]	N.S.	S.[f]	S	S

a elimination rate constant
b harmonic mean of half-life
c numbers in parenthesis represent the standard error of the mean
d female subject
e not significant (p=0.05)
f significant (p=0.05)

Excretion Rate

Pharmacokinetic parameters which have been utilized as a function of the rate of drug absorption are the peak serum concentration and the time necessary to reach the peak serum concentration (19). In a similar way the peak urinary excretion rate, as well as the time to reach that peak, have been used as suitable parameters to describe the rate of a drug absorption (14).

The average value of the urinary excretion rate was 54.2 mg/hr (range from 48 to 62 mg/hr), (Table 1), for all tested brands. The average value was the same (range from 42.2 to 64.7) when each subject was considered separately (Table 2). The average urinary excretion rate curves of the various brands are shown in Fig. 2. Analysis of variance indicated no significant differences ($p=0.05$) between brands and between subjects.

The average time at which the urinary excretion rate reached its peak for all brands and subjects are 2.16 hr (range from 2.0 to 2.3 hr), and 2.14 hr (range from 1.48 to 2.60 hr), respectively, (Tables 1 & 2). Analysis of variance for the time of peak renal excretion showed statistically significant differences ($p=0.05$) between the brands and between the subjects.

Half Life and Elimination Rate Constant

A one compartment model with first order absorption and elimination was used to calculate the elimination rate constants and the half lives of ampicillin, (Tables 1 & 2). The average elimination rate constant based on least-squares ananlysis for all brands and individual subjects are 0.485 hr^{-1} (range from 0.450 to 0.524 hr^{-1}),

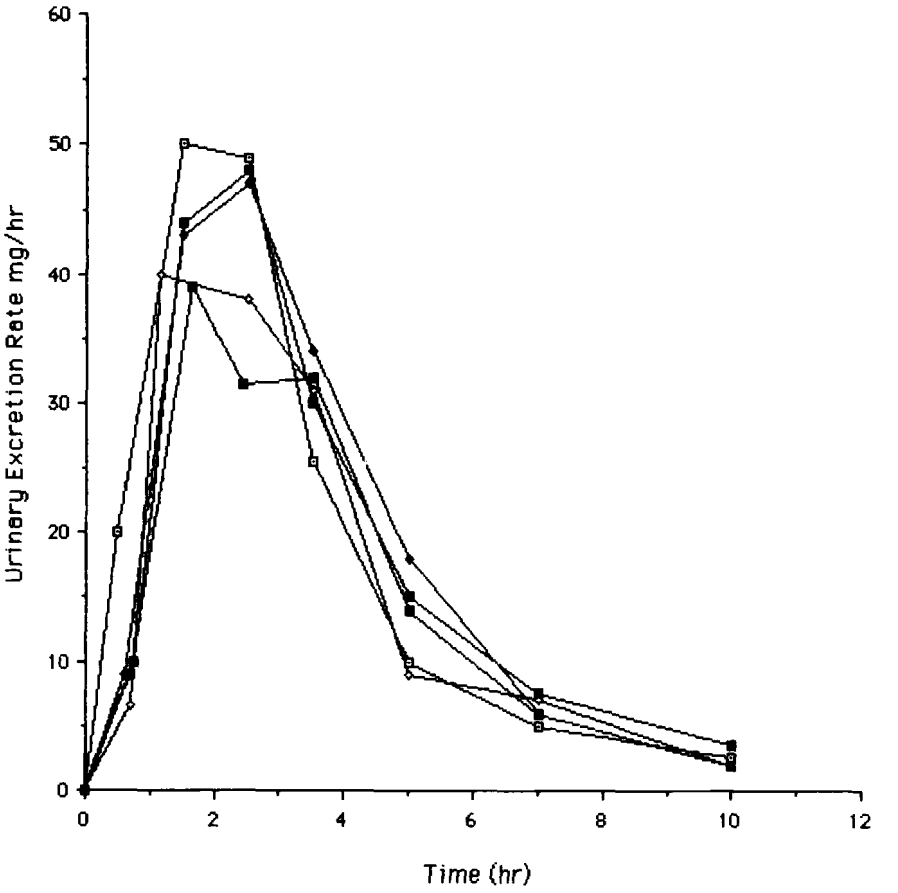

FIGURE 2

Average urinary excretion rates following oral administration of the following ampicillin dosage forms to twelve subjects: ⊡, brand A; ◆, brand B; ■, brand C; ◇, brand D; ■, brand E.

and 0.486 hr^{-1} (range from 0.411 to 0.549 hr^{-1}) respectively. While there were no significant differences (p=0.05) between different brands of ampicillin (Table 1), there were significant differences (p=0.05) between the subjects. The half life of the drug averaged 1.47 hr (range from 1.35 to 1.58 hr) between the brands (Table 1) and 1.48

TABLE 3
Relative Bioavailability of Different Tested Ampicillin Dosage Forms (Capsules and tablet) Following Oral Administration of 500mg of Ampicillin

volunteers	Brand (Capsule) A [a]	B [b]	C	D	E	Statistical Analysis
ME	100	57.5	63.3	67.1	85.1	S [c]
JA	100	103.5	142.8	113.6	130.9	S
DA	100	66.2	87.6	123.5	95.4	S
AM	100	81.5	137.2	85.0	88.3	S
GO	100	80.7	67.1	53.2	60.4	S
HO	100	105.9	93.4	53.8	38.0	S
NK+ [d]	100	107.3	138.7	122.6	58.8	S
MO+	100	189.2	93.1	154.6	115.7	S
SH+	100	133.0	121.4	143.4	100.6	S
SA+	100	90.1	74.0	166.1	123.4	S
HE+	100	97.0	106.2	154.2	81.4	S
KO+	100	135.6	63.0	118.2	96.5	S
Mean S.E.	100	103.9 (10.3)	99.0 (8.7)	112.9 (11.5)	89.5 (7.9)	
Statistical Analysis	N.S. [e]	N.S.	N.S.	N.S.	N.S.	

[a] used as standard
[b] the only tablet dosage form
[c] significant (p=0.05)
[d] female subject
[e] not significant (p=0.05)

TABLE 4

Average Pharmacokinetic Parameters for a Group of Male and Female Subjects (6 Subjects in Each Group) Following Oral Administration of 500mg Ampicillin in Different Dosage Forms (4 Capsules and One Tablet) to Twelve Subjects.

Volunteers	Average Cumulative Excreted (mg)	Urinary Peak height (mg/hr)	Time of Peaking (hr)	Ke [a] (hr^{-1})	T1/2 [b] (hr)
Male	183.7 (11.8) [c]	57.7 (2.5)	2.12 (0.29)	0.328 (0.091)	2.11
Female	169.3 (8.8)	50.7 (3.1)	2.16 (0.17)	0.502 (0.017)	1.38
Average	176.5 (7.3)	54.2 (2.4)	2.14 (0.10)	0.486 (0.016)	1.43

[a] elimination rate constant
[b] harmonic mean of half-life
[c] numbers in parenthesis represent the standard error of the mean

(range from 1.29 to 1.94 hr) between the individual subjects (Table 2).

Relative Bioavailability of Unchanged Ampicilllin

The cumulative amount of unchanged ampicillin excreted over a period of 8 hours after drug administration can be considered as a basis for calculating relative bioavailability. As it has been used throughout the study, using brand A as a standard (100% availability assumed), the relative bioavailability of all tested brands is shown in Table 3. No statistically significant differences (p=0.05) between the

TABLE 5

Average Relative Bioavailability of Different Tested Ampicillin Dosage Forms (Capsules and Tablet) For a Group of Male and Female Subjects (Six Subjects in Each Group) Following Oral Administration of 500mg of Ampicillin.

	Different Dosage Forms				
Volunteers	A	B[a]	C	D	E
Male	100	82.6(7.9)[b]	98.7(14.0)	82.7(12.3)	83.0(12.9)
Female	100	125.4(14.8)	99.4(11.7)	143.2(7.8)	96.1(9.6)
Average	100	103.9(10.3)	99.0(8.7)	112.9(11.5)	89.5(7.9)

[a] the only tablet dosage form

[b] numbers in parenthesis represent the standard error of the mean

different brands of ampicillin were observed. However significant intersubject variation was observed (Table 3).

Comparison between the Male and Female Subjects

It has been established that differences in sex as well as the other factors can significantly affect the intersubject variation in drug bioavailability (21). Mean pharmacokinetic parameters of different brands of ampicillin for male and female subjects in this study are shown in Tables 4 and 5 respectively. Using a student t-test, differences in bioavailability and other pharmakokinetic parameters, except for urinary excretion rate between the two different groups of male and female subject were not statistically significant.

DISCUSSION

Reports indicate that ampicillin absorption varies between 20-70% of the administered dose (15). Therefore, the bioavailability of the drug may be incomplete (22). According to the data in Table 1, an average of 35.5% (SE=1.5) of ampicillin was excreted during the 8 hours after the administration by the subjects. This value is in agreement with the data reported by Jusko and Lewis (18), i.e. 32 ± 8% and also Khalil et. al. (14), i.e. 27.8 ± 1.6. Statistical analysis of the data in this study indicates no significant differences between the different brands of ampicillin except for the time to reach the maximum urinary excretion.

Although the previous reports indicate considerable variability in ampicillin absorption between subjects following oral administration (1, 2). The present study showed no significant differences between the subjects in cumulative percentage of drug excreted after 8 hours calculated from Table 2. In fact, the percentage of ingested drug that was absorbed varied among individuals from 28.9 to 44.1% (average 35.3%). This amount agrees with the finding of Swahn (2), average of 44%, and Khalil et. al. (14) average of 27.8%.

Analysis of variance of the relative bioavailability of different tested brands of ampicillin indicates a significant difference ($p=0.05$) between the various subjects but not significant differences ($p=0.05$) between different brands.

Finally, it can be concluded that:

1. No significant differences between the tested ampicillin brands were observed.

2. No significant differences between male and female subjects were ovserverd.
3. There are significant differences between various subjects which can be expected.

The results of this study indicate that the behavior of different brands of ampicillin are compatible and bioequivalent.

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